IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Appellants	: FAN, Shihe; et al.	
Application Serial No.	: 10/726,574	Confirmation No. 5066
Filed	: December 4, 2003	
For	: METHOD OF EX VITRO SOWING, GERMINATION, GROWTH AND CONVERSION OF PLANT SOMATIC EMBRYOS OR GERMINANTS, AND NUTRIENT MEDIUM USED THEREFOR	
Art Unit	: 1661	
Examiner	: Annette H. PARA	

BRIEF ON APPEAL

This is Appellant's Brief under 37 C.F.R. §41.37 in support of Appellant's appeal to the Board of Patent Appeals and Interferences under 37 C.F.R. §41.31 from the final rejection of claims 1-10, 12-32 and 45 of the above-identified application.

(i) Real Party in Interest

The real party in interest is Cellfor Inc., a Canadian Corporation having a place of business in Vancouver, Canada.

(ii) Related Appeals and Interferences

There are no other appeals or interferences known to Appellant or Appellant's legal representative or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(iii) Status of Claims

Claims 1-10, 12-32 and 45 are currently in the application. All of these claims have been finally rejected under 35 U.S.C. §103(a) in an Examiner's Action dated February 8, 2002.

Claims 1-44 were present in the above-identified regular U.S. patent application filed December 4, 2003. Claims 33-44 were withdrawn from consideration following a requirement for restriction, and were cancelled without prejudice in an Amendment filed October 3, 2006 in response to an official action mailed on July 5, 2006. Claim 11 was also cancelled in the Amendment of October 3, 2006 in response to a rejection under 35 U.S.C. §112, and claim 25 was amended to correct an obvious clerical error. Claim 45 was added for the first time in a response filed (with a Request for Continued Examination) April 3, 2007 in response to a final action mailed on January 5, 2007. Claims 1, 32 and 45 were amended in an Amendment filed (with a second Request for Continued Examination and request for extension of time) October 31, 2007 in response to a final official action mailed June 11, 2007. The other claims are as originally filed. The claims appealed are all of the finally rejected claims, viz. claims 1-10, 12-32 and 45. All of the claims have been rejected on two or more occasions.

(iv) Status of Amendments

The aforementioned Amendment filed October 31, 2007 was entered. No amendment was filed subsequent to the final rejection (dated February 8, 2008) from which the present appeal is taken. The Examiner concluded: "Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made", and that "No claim is allowed" (page 6 of the Final Action). Appellant filed a Notice of Appeal (with a request for an extension of time) on August 6, 2008.

(v) Summary Claimed Subject Matter

In the following summary, page and line numbers refer to Appellant's specification, and Figure numbers and reference characters refer to the drawings of the above-identified application.

The claimed invention is directed to methods of sowing somatic plant embryos or germinants *ex vitro* in porous solid growth substrates, such a soil or peat, to allow the embryos or germinants to develop in non-sterile conditions into autotrophic seedlings and subsequently into mature plants (page 1, lines 6 to 9). The embryos or germinants are those of conifer plant species (claim 1, line 2). Merely by way of specific illustration, Figs. 1 through 5 show steps in the sowing of an embryo or germinant 20 into a nursery container 10, and the subsequent growth of the embryo or germinant into a seedling 28. In Fig. 1, shows how a slight depression 12 is formed in the surface of a porous solid growth substrate 11 (e.g. soil, peat or a soil-like product bound together with a polymer) contained in a container 10 forming part, for example, of a mini-plug tray (page 32, lines 8 to 15). In Fig. 2, a flowable or semi-solid nutrient medium 15 is dispensed onto the substrate 11 from dispenser 16. The medium 15 enters the depression 12 and forms a pool or body 18. The medium contains water and a carbohydrate nutrient for the embryo or germinant, but also contains a component, e.g. a gelling agent, that makes the medium flowable or semi-solid so that it does not become immediately absorbed into the porous substrate 11 but remains intact in the form of the pool or body. In addition, the medium contains a solid component in the form, for example, of fibers (claim 1). In Fig. 3, a somatic embryo or germinant 20 is sown in the container 10 in contact with the pool or body 18 of the medium with part exposed to air. Importantly, the medium provides support for the embryo or germinant 20 so that it remains in a desired upright position (page 32, lines 24 to 32). In Fig. 4, a period of time has passed during which a root 26 has commenced to grow from the embryo or germinant. Over this time period, the medium 15 has provided not only support to the embryo or germinant but also a source of water and carbohydrate nutrient to aid with its development. Eventually, though, the flowable or semi-solid component dissipates, but when this occurs, the solid component 25 remains in place around the embryo or germinant to

provide continued physical support to prevent the embryo, germinant or seedling from toppling over while the emerging root is in a juvenile condition (page 33, lines 5 to 11). Thus, the solid component of the medium is specifically chosen to have properties (e.g. an elongated fibrous form) such that it can remain in contact with the embryo or germinant after the flowable or semi-solid component has dissipated (claim 1). In Fig. 5, showing a condition after the passage of further time, the solid component 25 of Fig. 4 has become an indistinguishable part of the growth substrate (page 33, lines 13 and 14).

More particularly, the invention defined by the claims on appeal relates to such a method. The independent claims (claims 1, 32 and 45) all require the solid particles to be present in the nutrient medium in a concentration up to 10% (w/v). This ensures that the medium remains flowable so that it can be dispensed, e.g. as illustrated in Fig. 2, and so that the resulting nutrient medium is clearly distinguished from solid growth media of the kind onto which the embryos or germinants are sown because such growth media contain much higher contents of solid components. The claims also requires the embryo, as sown, to be exposed to environmental conditions effective for growth of the embryos or germinants into autotrophic seedlings. The claims further require the dispensing of the nutrient medium, the contacting of the embryos or germinants with the medium and the exposure to environmental conditions to be carried out *ex vitro* in non-sterile conditions. This shows that the method is not carried out in carefully controlled environments often used in the prior art for such an early transformation step, but rather that it is designed for an environment more commonly used for the planting of zygotic seeds, thereby making the method more economically acceptable and simple to operate.

The flowable component of the nutrient medium may be a fluid comprising a viscous liquid containing the carbohydrate nutrient in dissolved form (claim 18) or a semi-solid material comprising a flowable gel (claim 19). Such materials are preferably chosen to impart a viscosity effective to cause at least some of the flowable component to remain in contact with the embryo or germinant until the embryo or germinant commences autotrophic growth (claim 2), or to have a cohesiveness (claim 32) such that the fluid or semi-solid remains in contact with the embryo or germinant under the environmental conditions employed. By making the

viscosity or cohesiveness quite high, for example, the flowable component may have lower tendency to dissipate and thus lasts longer in contact with the embryo or germinant. By continuing such contact to the autotrophic phase of growth, the beneficial effect of the medium is prolonged to the point at which the seedling can develop further without such help.

Ideally, the fluidity of the medium is made such that it can be dispensed under gravity or pressure from an orifice (claim 3) so that the type of dispensing shown in Fig. 2 is facilitated. The embryo or germinant is preferably contacted with the nutrient medium after the medium has been dispensed, as shown in Figs. 2 and 3 (claim 4), e.g. to form a pool (claim 45), but the contact could be made before (claim 5) or simultaneously with such dispensing step (claim 6). Preferably, part of the embryo or germinant remains uncoated (claim 7) and the nutrient medium is dispensed into a depression in the growth substrate as shown in Fig. 1 (claim 8), and may be dispensed in contact with the embryo or germinant (claim 9). The embryo or germinant may be naked (claim 10) when contacted with the nutrient medium, which is particularly advantageous because the embryos or germinants do not then require any coating or covering treatment.

The solid particles in the nutrient medium are preferably biologically inert (claim 29) and preferably elongated and may comprise flexible fibers, e.g. made of alpha-cellulose (claims 12, 13 and 14), or may be particles of a variety of solids (claim 16). A gelling agent may be used in the nutrient medium to make it flowable, and any one of a variety of gelling agents may be used (claim 15). The nutrient medium may additionally contain other kinds of ingredients useful for the embryo or germinant (claim 20), and the carbohydrate is preferably a monosaccharide (claim 21) such as glucose or fructose (claim 22), or an oligosaccharide (claim 23) such as sucrose, maltose, raffinose or stachyose (claim 24). A combination of such carbohydrates may be used (claim 25). The carbohydrate nutrient is preferably present in the nutrient medium in an amount of 1 to 10% (w/v) (claims 27 and 28).

The embryos or germinants are preferably from the family Pinaceae (claim 30), such as Loblolly pine, Radiata pine, Spruce and Douglas fir (claim 31).

(vi) Grounds of Rejection to be Reviewed on Appeal

The issues presented for review on this appeal are whether:

(a) claims 1-10, 12-20, 23-24, 27, 29-32 and 45 are unpatentable under 35 U.S.C. §103(a) over Fan et al. (United States Patent No. 6,444,467) in view of Pierik (*In Vitro Culture of Higher Plants*, 1997); and

(b) claims 21, 22, 25, 26 and 28 are unpatentable under 35 U.S.C. §103(a) over Fan et al. in view of each of Gupta (United States Patent 5,563,061) and Tremblay et al. (Plant Cell, Tissue and Organ Culture **42**:39-46 1995).

(vi) Argument

A. Errors in the Rejection

It is submitted that the rejection of claims 1-10, 12-20, 23-24, 27, 29-32 and 45 as unpatentable under 35 U.S.C. §103(a) over Fan et al. in view of Pierik is in error in that the steps set forth in the independent claims (claims 1, 32 and 45) would not have been obvious from the applied references, taken together, and however combined, at the time the invention was made.

It is submitted that the rejection is in error in that certain elements required by the independent claims are missing from the cited references. In particular, the provision of "nutrient medium comprising particles of a solid component contained within a flowable component containing water and a carbohydrate nutrient ... said flowable component being selected from ... a fluid and a semi-solid, and said solid particles being present at a concentration of up to 10% (w/v)" (e.g. claim 1) is not disclosed nor made obvious. When the cited references disclose any material for contact with embryos, it is either a growth substrate comprising mostly solids or a solids-free liquid for supplying a nutrient. The particular nutrient

medium required by Appellant's claims is unobvious absent the benefit of hindsight when the advantages of prolonged water and nutrient supply to the embryos or germinants, together with even longer physical support provided by the medium become apparent.

Furthermore, in the case of claim 45, there are additional requirements that are not disclosed in the prior art. These additional requirements are that the nutrient medium (containing a semi-solid component) be dispensed to form a pool on the surface of the growth medium, and that the embryos or germinants be contacted with the nutrient medium in such a manner that the pool provides the embryos or germinants with physical support to maintain a generally upright growth orientation.

It is furthermore submitted that the rejection of claims 21, 22, 25, 26 and 28 as unpatentable under 35 U.S.C. §103(a) over Fan et al. in view of each of Gupta and Tremblay et al. is in error. Firstly, these claims are dependent claims and they are patentable for the same reasons as the claims from which they depend. Additionally, however, the Examiner has not recognized that somatic embryogenesis involves several steps that are quite different from each other and has cited features from earlier steps of somatic embryogenesis against the claims of the present application which are confined to a final step of converting embryos or germinants into autotrophic seedlings. A person of ordinary skill in the art would recognize that features or materials designed for one step of somatic embryogenesis would not likely be effective for another step, and this is particularly so when comparing early steps (e.g. embryo proliferation or maturation) with the last step (conversion to seedlings). The subject matter of the rejected claims is therefore unobvious for this additional reason.

B. Claim Limitations Not Described in the Prior Art

None of the references describe the steps of providing a nutrient medium comprising particles of a solid component contained within a flowable component containing water and a carbohydrade nutrient, the flowable component being a fluid and a semi-solid, and the solid particles being present in a concentration up to 10% (w/v), dispensing a quantity of the nutrient medium onto a surface of a porous solid growth substrate, and contacting a somatic plant embryo or germinant with the nutrient medium. There is no disclosure of the use of particles

of a solid component in a nutrient medium being used to provide enduring physical support for germinants and seedlings once a flowable component thereof has dissipated. Additionally, when considering claim 45, none of the cited references disclose the provision of a pool of nutrient medium to hold and maintain embryos or germinants in a generally upright position for proper growth.

C. How Such Limitations Render the Claimed Subject Matter Unobvious over Fan et al., Pierik, Gupta and Tremblay et al., however combined.

In the following discussion, statements and assertions attributed to the Examiner can be found in the final rejection of February 8, 2008 and page numbers refer to pages in that document.

C.1 Rejection of claims 1-10, 12-20, 23-24, 27, 29-32 and 45 as unpatentable under 35 U.S.C. §103(a) over Fan et al. in view of Pierik.

The broadest of the rejected claims are directed to a method of sowing comprising the above steps together with the step of exposing the embryo or germinant to environmental conditions effective for their growth into autotrophic seedlings. The steps of dispensing, contacting and exposing are carried out *ex-vitro* in non-sterile conditions, and the particles are such that they are adapted to remain in contact with the embryo or germinant after the flowable component has dissipated in the environmental conditions, thereby providing continuing physical support for the embryo or germinant after the dissipation.

The Examiner asserts that Fan et al. teach a nutrient medium comprising 1-9% of sucrose, solid, liquid and gas phases for growth of somatic embryos into autotrophic seedlings. The Examiner further asserts that the solid components are, but are not restricted to, vermiculite, perlite, peat (pulp of wood containing alpha cellulose), coconut husk fibers (which are flexible fibers) and the like. The embryos are held on the surface or above the surface of the medium by the means of a physical support such as polypropylene materials. The Examiner further asserts that Fan et al. teach sowing somatic embryos in mini-plug trays having their drainage holes covered by a mesh-like material to support the somatic embryos, and that the

containers are then placed onto liquid germination media such that the embryos are in contact with but are not submerged in the liquid media. The Examiner asserts that Fan et al. teach a solid component comprising elongated particles and teach the growing of somatic embryos of *Pinus radiata, Pinus taeda* (Loblolly pine), and *Picea glauca* (Spruce), which are conifer species.

The Examiner further asserts that the nutrient medium used by Fan et al. has a viscosity, and remains in contact with the embryos. Further, when the somatic embryo is sown onto the surface of the absorbent material it creates a depression in the solid surface providing physical support for the embryos. Fan et al. suggest pouring a nutrient solution onto the three-phase substrate prior to sowing of the pre-germinated somatic embryos to adjust the moisture content of the substrate to 60-85%. The medium in contact with the pre-germinated embryos is made of three-phase substrate and nutrient solution.

The Examiner finally asserts that Fan et al. teach sowing the pre-germinated embryos onto three-phase media which are drenched with the nutrient solution.

The Examiner comments that the teaching of Fan et al. differs from the claimed invention in that (a) Fan et al. fail to teach a nutrient medium comprising gelling agents; and (b) Fan et al. fail to teach the content of solids in the medium to a maximum of 10%. The Examiner then cites Pierik as disclosing these missing elements.

Before discussing Pierik, it is pointed out that the Examiner's analysis of Fan et al. is incorrect and/or relies on features picked from Fan et al. without consideration of the context in which they are presented.

Fan et al. disclose a multi-step process to produce seedlings from somatic embryos. The first main step involves "pre-germination" followed by placing the embryos into a state of physical dormancy (Col. 3, lines 43 to 51). The second main step involves placing the pre-germinated embryos developed in this way on or within the surface of a three-phase substrate (e.g. soil or soil-substitute - the phases being solid liquid and gas), and then transferring the substrate containing the pre-germinated embryos into an environmentally-controlled plant growth environment and applying water and/or nutrient solutions at regular intervals (e.g. by misting with micro-droplets or by drenching - Col. 3, line 60 to Col. 4, line 7 and Col. 11, lines 5 to 10). The claims of the present invention have nothing to do with "pre-germination", i.e. the

first step, and are limited to the step of sowing the embryos or germinants for their transformation into seedlings, therefore any disclosure in Fan et al. that relates to the pre-germination step is irrelevant to the present invention. Pre-germination is preferably carried out *in-vitro* in sterile conditions (Col. 5, lines 44-46) and is preferably followed by the step of placing the embryos into a state of physical dormancy, e.g. by desiccation (Col. 4, lines 46 and 47). The Examiner seems to have overlooked this distinction. For example, the Examiner states (page 2) that:

> "The solid components are but not restricted to vermiculite, perlite, peat (pulp of wood containing alpha cellulose), coconut husk fibers (which are flexible fibbers) and the like".

In support of this, the Examiner cites Column 8, lines 50-51 of Fan et al., but this part of Fan et al. describes the pre-germination step. The passage at Col. 8 lines 48 to 54 reads:

> "Alternatively, the somatic embryos can be successfully <u>pre-germinated</u> on discontinuous physical substrates comprised of materials such as but not limited to, vermiculite, perlite, peat, coconut husk fibers and the like, said discontinuous supports containing sufficient <u>liquid germination medium</u> to enable the formation of a thin capillary layer or film of <u>germination medium</u> around the somatic embryos." (underlining added)

The Examiner also referred to a section of Fan et al. describing the pre-germination step, when saying (page 2/3):

> "The somatic embryos are held on the surface or above the surface of the medium by the means of a physical support such as polypropylene materials (column 8, lines 43-44)."

The passage in question (Col. 8, lines 40 to 48) reads:

> "It is preferable that the <u>pre-germination step</u> is carried out in a container wherein there is a physical support such as, but not restricted to, filter paper or a screen comprised of nylon or polypropylene material or other such materials, is placed on the liquid <u>germination medium</u> such that plant somatic embryos are held on the surface or above

> the surface of the liquid medium such that a thin capillary layer or film of the germination medium is formed around the somatic embryos." (underlining added)

Not only does this clearly relate to pre-germination, but the objective is to provide a thin capillary layer or film of liquid around the embryo. Such a layer would not last long in an environment provided for non-sterile *ex-vitro* sowing and conversion to seedlings.

The Examiner makes the same mistake in making a statement (page 3) based on Col. 8, lines 59-67 of Fan et al. that:

> "Fan et al. also teach sowing somatic embryos in mini plug trays having their drainage holes covered by a mesh-like material to support the somatic embryos. These containers are then placed onto liquid germination media such that the embryos are in contact with but are not submerged in the liquid media (column 8, lines 59-67)."

The passage in question (Col. 8, line 59 to Col. 9, line 2) reads as follows:

> "However, it is also possible to accomplish the <u>pre-germination</u> of somatic embryos by sowing them with conventional seeding equipment into empty multi-chambered nursery containers exemplified by but not restricted to miniplug trays, said containers having their drainage holes covered by a mesh-like material which will support the somatic embryos after sowing. The containers are then placed onto liquid <u>germination media</u> such that the somatic embryos are in contact with but are not submerged in the liquid media, such that a thin layer of film of germination medium is formed around the somatic embryos." (underlining added)

Similarly, the Examiner refers (page 3) to this part of Fan et al. to support the interpretation that:

> "When the somatic embryo is sown onto the surface of the absorbent material (column 8, lines 55-58) it creates a depression in the solid surface providing physical support for the embryos."

The passage in question reads:

> "The somatic embryos can be sown onto the surface of the absorbent material by hand or by the means of a mechanical sowing device such as but not restricted to conventional seeding equipment."

Again, this relates to pre-germination (see Col. 8, lines 48 and 49, which set the context for the above passage). Moreover, the Examiner's statements regarding the formation of a depression and the provision of physical support are not mentioned in this passage and appear to be speculative and not shown in the cited document.

In case it may be argued that sowing for pre-germination is the same as sowing for growth into seedlings, it should be kept in mind that the broad claims of the present application require "exposing said embryo or germinant to environmental conditions effective for growth into an autotrophic seedling". This is clearly not done during pre-germination (because the embryos are to be converted to autotrophic seedlings by Fan et al. only in a later step). Moreover, as the passages quoted above show, the intention during pre-germination is to cover the surfaces of the embryos with a capillary layer or film of liquid medium, whereas, in the broad claims of the present application, the intention is to contact the embryos or germinants with a quantity of nutrient medium dispensed onto the surface of a growth substrate, which may not result in the formation of a capillary layer of liquid around the embryos, and is likely not to do so.

By choosing to rely heavily on statements from Fan et al. that describe the pre-germination step rather than the step of sowing for growth into autotrophic seedlings, the overall teaching of Fan et al. has been distorted. Fan et al. do describe a step of sowing for growth into seedlings, but this step does not bear any resemblance to the steps required in the broad claims of the present application. For example, in Col 9, lines 12 to 16, Fan et al. state:

> "Another important advantage of the present invention, at least in its preferred forms, is that the sowing and propagation of pre-germinated somatic embryos can be practiced with a wide variety of non-sterilized growing substrates commonly used in conventional plant propagation."

After describing various kinds of conventional growing substrates, Fan et al. continue at Col. 9, line 64 to Col. 10, line 5:

> "After the pre-germinated somatic embryos are sown onto the surfaces of the rooting substrates, if desired, the embryos may be covered with a thin layer of additional rooting substrate that may be comprised of the same material underneath the embryos or alternatively, with a different type of material. One non-limiting example is sowing the pre-germinated embryos onto PRO-MIX-PGX medium, then overlaying the embryos with a thin layer of coconut husk fibres."

This is very much like a conventional process of planting seeds and then covering them with a thin layer of soil. There is no suggestion that the covering layer should contain a flowable component selected from fluid or semi-solid, and clearly, in the procedure of Fan et al. the solid particles would often be present in an amount approaching 100% rather than 10% or less required by the claims of the present application.

At Col. 10, lines 45 to 50, Fan et al. state:

> "Another important feature of the invention, at least in preferred forms, is that the exogenous nutrients, including but not restricted to carbohydrates and minerals, required for successful somatic embryo re-germination and subsequent growth and development may be applied as aerosols. The nutrient solutions may be applied with, but not restricted to, conventional misting and/or fogging equipment."

It is clear from this that Fan et al. did not contemplate incorporating a carbohydrate nutrient into a nutrient medium contacted with the embryos during sowing. Instead, the nutrient is applied later in a solution thin enough to be subjected to misting or fogging. Nutrient solutions applied in this way would clearly dissipate very quickly. In contrast, the nutrient medium of the broad claims of the present application is intended to persist as the embryo or germinant develops into a seedling. In Fan et al., an alternative to such misting or fogging is to irrigate or "drench" the growth substrate with nutrient solution just before the embryos are sown in the growth substrate (Col. 11, lines 6 to 12). The nutrient solution supplied to the growth substrate in this way contains no solid component that may provide support for the embryos and seedlings once the liquid has dissipated.

In summary, not only do Fan et al. fail to disclose a nutrient solution containing gelling agents and a content of solids to a maximum of 10% (as acknowledged by the Examiner), they

also fail to disclose the step of dispensing a quantity of nutrient medium (containing a solid particles) onto a surface of a porous solid growth substrate and contacting the embryos with the nutrient medium. Moreover, there is no disclosure of solid particles from the nutrient medium remaining to provide continued physical support for the seedlings after dissipation of the other components of the medium.

The Examiner relied on Pierik to overcome the deficiencies of Fan et al. The Examiner stated: "Pierik teaches nutrient media comprising agar to form a gel (page 55)". It is to be noted that Pieirik is entitled: "In Vitro Culture of Higher Plants" (see title page). It is therefore clearly concerned only with tissue culture *in vitro*. This is reinforced by the statement on page 56 at lines 7 and 8 that: "In vitro growth may be adversely affected if the agar concentration is too high." The broad claims of the present application specifically require the steps to be carried out *ex-vitro*. It would seem that Pierik is concerned with the type of culture steps that take place on solid gelled media in sterile conditions, e.g. the type of propagation and maturation steps that are typically carried out in the earlier phases of somatic embryo development. Pierik states on page 56 at lines 1 to 6 that:

> "The usual concentration for agar is 0.6-0.8%. If a lower concentration (0.4%) is used then the nutrient medium remains sloppy, especially when the pH is also low. If a high concentration (1.0%) is chosen, then the nutrient medium is very solid, making inoculation difficult. If 0.6% is used and the medium remains sloppy then the pH should be corrected; if the pH is lower than 4.5-4.8 a medium with 0.6% agar does not gel properly."

The implication of this passage is that the nutrient medium should range in solidity between being too sloppy and very solid. This teaches away from the use of a "flowable" medium as required by the claims of the present invention.

The Examiner stated (page 3) that:

> "The percentage of solids in the medium is an optimization of parameters. The reference does not specifically teach a content of 10% solids as claimed by Applicant. The percentage of solids in the medium is a clearly result effective parameters that a person of ordinary skill in the art would routinely optimize."

The Examiner seems to be confusing the requirement for 10% solids in the claims of the present application with the amount of agar used in gelled media of the kind described by Pierik. However, these are two different things. The nutrient medium of the present invention contains particles of a solid component (such as fibers of alpha-cellulose) and (at least in preferred embodiments) a gelling agent such as agar, gellan gum, etc. (claim 15) which is regarded (in the concentrations employed) as a semi-solid. On the other hand, the media of Pierik do not contain particles of a solid of any kind. It is stated on page 55 line 6 that solubilized agar may form a gel that can "absorb compounds", but this presumably means that it can absorb compounds in solution. There is no reference to the incorporation of solid particles into the gel. Indeed, since the medium is intended to provide nutrients to tissues, it is difficult to see how solid particles would make a contribution to this. In the embodiments of the present application, the solid particles are employed in order to provide physical support for the seedlings. Therefore, contrary to the suggestion of the Examiner, a person of ordinary skill in the art, upon reading Pierik, would not see the need to optimize the solids content to a maximum of 10% as a routine optimization of Pierik, because Pierik does not suggest the use of any solids whatsoever.

The Examiner continued (page 3/4):

> "It would have been customary for an artisan of ordinary skill to determine the optimal concentration of solids to help the nutrient to stay in contact with the embryos, thus giving more usefulness to the method. One would also have been motivated to use this percentage of solids in the medium to optimize the surface of the embryos in contact with the nutrient. Thus, absent some demonstration of unexpected results from the claimed parameters, the optimization of the content of 10% solids in the medium would have been obvious at the time of Applicant's invention."

As noted, since Pierik discloses no content of particles of a solid, this statement makes no sense. Again, there is apparent confusion between the gelling agent and the particles of a solid component.

The Examiner then said (page 4):

> "At the time the invention was made it would have been obvious for one of ordinary in the art to modify the method of Fan et al. by adding agar mixed with the nutrient medium knowing that gelling agent serve as binding agent for nutrient and water and to use 10% of solids. One of ordinary skill in the art would have been motivated to do that because adding this step will lower the maintenance of the germinant as no added water or nutrient will be needed because they will be contained in the gelling agent and also because the low percentage of solids in the medium will raise the surface of embryos in contact with the nutrient."

The statement that "the low percentage of solids in the medium will raise the surface of the embryos in contact with the nutrient" seems to have no bearing on Pierik, Fan et al. or the present invention and, to the extent that it can be understood, is wrong. The surfaces of the embryos are not raised by the solids in the medium. In the claimed invention, the solids in the medium provide support for the embryos or germinants.

Considering the teachings of Fan et al. and Peirik objectively, there is no reason why a person of ordinary skill in the art would consider them to be related. Pierik is concerned with *in-vitro* tissue culture. After the pre-germination step, Fan et al. are concerned with growing the embryos into seedlings *ex-vitro*. They do this by transferring the embryos to a growth substrate, possibly covering the embryos with the same or another growth substrate, and then expose the embryos to growth conditions. Nutrient medium is either supplied by periodic misting or fogging, or by a preliminary drench of the growth substrate prior to sowing. The use of a gelling agent in the nutrient medium in either of these methods would be counter-productive or impossible. A gelling agent would make misting or fogging impossible. A gelling agent in the medium intended for drenching would make the medium unsuitable for drenching or would form a gel within the growth substrate. Neither procedure (misting/fogging or drenching) could be expected to be improved by the incorporation of a gelling agent or particles of a solid and, even if this were not the case, there would still be a missing element, i.e. there would be nothing to suggest that the nutrient medium should be made to contain particles of a solid component in an amount of up to 10% in order to provide continuing physical support for the seedling after dissipation of the other components of the medium.

In short, Pierik does not serve to cure the deficiencies of Fan et al. It is submitted that the Examiner has failed to establish a *prima facie* case of obviousness based on the factual inquiries laid out in Graham v. John Deere Co., 148 USPQ 459 (1966). As highlighted in MPEP 2142:

> "The Examiner bears the initial burden of factually supporting any *prima facie* conclusion of obviousness. If the Examiner does not produce a prima facie case, the applicant is under no obligation to submit evidence of non-obviousness."

In the present case, particularly in view of the misinterpretation of the teaching of both Fan et al. and Pierik, and the absence of claimed elements as outlined above, it is submitted that the Examiner has relied upon a faulty line of reasoning to justify the obviousness rejection.

Accordingly, it is believed that the rejection of the indicated claims over Fan et al. in view of Pierik is in error and should be reversed.

C.2 Rejection of claims 21, 22, 25, 26 and 28 as unpatentable under 35 U.S.C. §103(a) over Fan et al. in view of each of Gupta and Tremblay et al.

The Examiner further rejected claims 21, 22, 25, 26 and 28 as unpatentable over Fan et al. in view of each of Gupta and Tremblay et al. (page 4). The rejected claims relate to the identity and quantity of the carbohydrate nutrient employed in the nutrient medium. These claims are dependent claims and they are dependent directly or indirectly on claim 1. The Examiner stated that the teaching of Fan et al. differs from the claimed invention as follows (page 4):

> "Fan et al. fail to teach the use of monosaccharides in the nutrient medium. Fan et al. fail to use glucose or fructose as carbohydrate. Fan et al. also fail to teach maltose as a carbohydrate nutrient. Finally Fan et al. fail to teach the content of solids in the medium to a maximum of 10%."

The Examiner omitted to mention here that Fan et al. also fail to teach a nutrient medium comprising gelling agents (as previously admitted by the Examiner).

The teaching of Fan et al. has been discussed extensively above. In particular it is submitted that the use of particles of a solid component up to 10% in the nutrient medium is unobvious from Fan et al. even in consideration with the teaching of Pierek. For this reason and others, it is believed that claim 1 of the present application is unobvious from Fan et al. Since claims 21, 22, 25, 26 and 28 are dependent directly or indirectly from claim 1, it is believed that these claims are also unobvious for the same reasons as claim 1. In particular, Gupta and Tremblay et al. do not suggest the use of particles of a solid component up to 10% in a nutrient medium and thus do not compensate for the lack of teaching of Fan et al. in this regard. Indeed, Gupta and Tremblay et al. were not cited by the Examiner for this purpose. On the contrary, Gupta was cited to show the use of 3% maltose as a carbohydrate nutrient in embryo culture, and Tremblay et al. was cited to show the use of monosaccharides (glucose and fructose), oligosaccharides and combinations thereof in a nutrient medium.

It repeated that the claims of the present application relate only to the step of transforming somatic embryos or germinants into autotrophic seedlings (see claim 1, lines 1 and 2). As disclosed by Gupta (see, for example, the Abstract), there are several steps in somatic embryogenesis and the conversion of embryos into plants. These steps include the excision of suitable tissue, the generation of embryogenic tissue, the propagation of embryogenic cells, the conversion into embryos and the maturation of such embryos, and then (optionally) pre-germination, desiccation and storage of the mature embryos. Finally, the mature embryos are converted into seedlings. The steps up to and including desiccation generally take place in sterile conditions using *in-vitro* procedures. Each step generally requires the use of a specific medium for propagation, maintenance, culturing, transformation, etc. and the ingredients of the medium (as well as environmental conditions) are carefully selected and controlled to ensure that the requirements and goals of the particular step are achieved. Accordingly, ingredients chosen for one step in such a procedure may not be suitable for some or all other steps, and may indeed be harmful. For this reason, the Examiner's reliance on Gupta and Tremblay et al. is unsound because these references do not relate to the step to which the claims of the present invention relate, i.e. the sowing and transformation of embryos or germinants into autotrophic seedlings.

As its title shows, Tremblay et al. relates to the maturation of black spruce somatic embryos. Maturation is the step of culturing immature embryos in a suitable medium to allow the embryos to develop the internal resources and possibly external characteristics necessary to undergo germination in a subsequent step. Tremblay et al. investigated the effects of sucrose, fructose and glucose on embryo maturation. Each of fructose and glucose produced fewer mature embryos (Abstract, line 7) compared to sucrose, thus teaching away from the use of these carbohydrates in the Tremblay et al. operations. Moreover, the article concluded that "the action of sucrose on embryo maturation is mostly achieved through an osmotic control" (Abstract, line 12). In other words, sucrose is not acting as a nutrient and acts instead to generate osmotic pressure by physical means. Therefore, even if a person of ordinary skill in the art would think of using a medium developed for embryo maturation as a nutrient medium for transformation of embryos or germinants into seedlings (which Appellant asserts would not be the case), the results of Tremblay et al. would discourage or teach away from the use of sucrose (and even more so, glucose and fructose) as a nutrient for the medium.

Gupta teaches the use of maltose as a carbon and energy source in the maintenance and multiplication cultures of somatic embryos (see Abstract, line 18). Gupta concludes: "The use of maltose at earlier stages of embryo development is more important than its use for embryo maturation" (Abstract, lines 21 to 23). This shows that (a) Gupta is concerned only with particular stages of embryo development up to embryo maturation, and (b) that maltose was more effective for embryo development than for embryo maturation, thus showing that a component effective for one stage may not be preferred for another stage. It is to be noted that, while Gupta mentions germination before or after storage and transplanting to soil for further growth (Abstract, lines 16 and 17), there is no suggestion by Gupta of the use of maltose for such steps.

It is therefore submitted that a person of ordinary skill in the art would not see either one of Tremblay et al. and Gupta as relevant to the transformation of mature embryos or germinants into seedlings, and thus would not see Tremblay et al. and Gupta as relevant to the present invention.

SEPARATE PATENTABILITY OF CLAIM 45

It is noted that the Examiner did not specifically comment on the limitations in claim 45 that are not present in claims 1 and 32, i.e. that the nutrient medium forms a pool and the embryo or germinant is contacted with the pool to provide the embryo or germinant with physical support to maintain a generally upright growth orientation after sowing. These features are missing from all of the cited prior art references and are unobvious therefrom as there is no suggestion of providing support for the embryos at this stage of their sowing.

(viii) Claims Appendix

A copy of the claims on appeal is set forth in an Appendix immediately following the conclusion and signature page below, and is incorporated herein by this reference. These claims show the amendments made during prosecution by the usual means.

Conclusion

For the foregoing reasons, it is respectfully requested that the decision of the Examiner rejecting claims 1-10, 12-32 and 45 be reversed, and that the claims be allowed.

Respectfully,

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(ix) Appendix

1. A method of sowing a heterotrophic somatic plant embryo or germinant of a conifer species to facilitate growth of the embryo or germinant into an autotrophic seedling, which method comprises the following steps:

providing a nutrient medium comprising particles of a solid component contained within a flowable component containing water and a carbohydrate nutrient for the embryo or germinant, said flowable component being selected from the group consisting of a fluid and a semi-solid, and said solid particles being present at a concentration up to 10% (w/v);

dispensing a quantity of the nutrient medium onto a surface of a porous solid growth substrate for the somatic plant embryo or germinant and contacting said plant embryo or germinant with said nutrient medium; and

exposing said embryo or germinant to environmental conditions effective for growth into an autotrophic seedling;

wherein at least said dispensing, contacting and exposing steps are carried out *ex vitro* in non-sterile conditions; and

wherein said particles forming said solid component are adapted to remain in contact with said embryo or germinant after said flowable component undergoes dissipation in said environmental conditions, thereby providing continuing physical support for said embryo or germinant after said dissipation.

2. The method of claim 1, which comprises employing, as said flowable component, a material having a viscosity that causes at least some of said flowable component containing the carbohydrate nutrient to remain in contact with said embryo or germinant until said embryo or germinant commences autotrophic growth under said environmental conditions.

3. The method of claim 1, which comprises employing, as said nutrient medium, a medium that has a fluidity under said environmental conditions that enables the nutrient medium to be dispensed under gravity or pressure from an orifice onto said porous solid growth substrate.

4. The method of claim 1, wherein said somatic plant embryo or germinant is contacted with said nutrient medium after said nutrient medium has been dispensed onto said surface of the porous growth substrate.

5. The method of claim 1, wherein said somatic plant embryo or germinant is contacted with said nutrient medium before said nutrient medium has been dispensed onto said surface of the porous growth substrate.

6. The method of claim 1, wherein said somatic plant embryo or germinant is contacted with said nutrient medium as said nutrient medium is dispensed onto said surface of the porous growth substrate.

7. The method of claim 1, wherein said somatic plant embryo or germinant is contacted with said nutrient medium in such a way that part of said somatic plant embryo or germinant remains uncoated with said nutrient medium.

8. The method of claim 1, wherein said porous solid growth substrate is in the form of a body provided with a depression formed in said surface, and wherein said nutrient medium is dispensed into said depression.

9. The method of claim 8, wherein said embryo or germinant is at least partially inserted into said depression in contact with said nutrient medium.

10. The method of claim 1, wherein said embryo or germinant, when contacted with said nutrient medium, is naked.

11. Canceled.

12. The method of claim 1, wherein said solid component of said nutrient medium comprise elongated particles.

13. The method of claim 12, wherein said elongated particles comprise flexible fibers.

14. The method of claim 13, wherein said fibers are made of alpha-cellulose.

15. The method of claim 14, wherein said nutrient medium contains methylcellulose, agar, agarose, phytagel, gellan gum or gelcarin either singly or in combinations of two or more of the aforementioned gelling agents.

16. The method of claim 1, wherein said solid component of said nutrient medium comprises milled or sifted peat moss, perlite, vermiculite, clay, diatomaceous earth, coir or silica either singly or in combinations of two or more of the aforementioned components.

17. The method of claim 16, wherein said nutrient medium contains methylcellulose, agar, agarose, phytagel, gellan gum or gelcarin either singly or in combinations of two or more of the aforementioned gelling agents.

18. The method of claim 1, wherein said flowable component of said nutrient medium is a fluid comprising a viscous liquid containing said carbohydrate nutrient in a dissolved form.

19. The method of claim 1, wherein said flowable component of said nutrient medium is a semi-solid comprising a flowable gel containing said carbohydrate in dissolved form.

20. The method of claim 1, wherein said nutrient medium additionally comprises at least one material selected from the group consisting of mineral compounds, vitamins, amino acids, plant growth regulators and pest control compounds.

21. The method of claim 1, wherein said carbohydrate nutrient is a monosaccharide.

22. The method of claim 21, wherein said monosaccharide is selected from the group consisting of glucose and fructose.

23. The method of claim 1, wherein said carbohydrate nutrient is an oligosaccharide.

24. The method of claim 23, wherein said oligosaccharide is selected from the group consisting of sucrose, maltose, raffinose, and stachyose.

25. The method of claim 1, wherein said carbohydrate nutrient is a combination of monosaccharides, a combination [[or]] <u>of</u> oligosaccharides or a combination of monosaccharides with oligosaccharides.

26. The method of claim of 25, wherein said monosaccharides are selected from the group consisting of glucose and fructose, and said oligosaccharides are selected from the group consisting of sucrose, maltose, raffinose, and stachyose.

27. The method of claim 1, wherein said carbohydrate nutrient is sucrose present in the nutrient medium in an amount in the range of in the range 1 and 10% (w/v).

28. The method of claim 1, wherein said carbohydrate nutrient is maltose present in the nutrient solution in an amount in the range of in the range 1 to 10% (w/v).

29. The method of claim 1, wherein said solid component is biologically inert.

30. The method of claim 1, wherein said embryo or germinants are from the family Pinaceae.

31. The method of claim 1, wherein said embryo or germinants are of tree species selected from the group consisting of Loblolly pine, Radiata pine, spruce and Douglas fir.

32. A method of growing an autotrophic seedling from a somatic plant embryo or germinant of a conifer species, which method comprises the following steps carried out *ex vitro* in non-sterile conditions:

providing a nutrient medium comprising particles of a solid component present within a flowable component, said flowable component being selected from the group consisting of an aqueous fluid and an aqueous semi-solid, containing a carbohydrate nutrient for the embryo or germinant, and said solid particles being present at a concentration up to 10% (w/v);

dispensing a quantity of the nutrient medium onto a surface of a porous solid growth substrate for the somatic plant embryo or germinant held in a container;

contacting said plant embryo or germinant with said nutrient medium; and

subjecting said embryo or germinant to environmental conditions to further growth and development into an autotrophic conifer seedling;

wherein said nutrient medium has a cohesiveness such that at least some of said fluid or semi-solid containing the carbohydrate nutrient remains in contact with said embryo or germinant as said embryo or germinant proceeds to develop under environmental conditions effective for said development; and

wherein said particles of the solid component are adapted to remain in contact with said embryo or germinant after of said flowable or semi-solid material dissipates under said environmental conditions, thereby providing continuing physical support for said embryo after said dissipation.

Claims 33 – 44: Canceled.

45. A method of sowing a heterotrophic somatic plant embryo or germinant of a conifer species to facilitate growth of the embryo or germinant into an autotrophic seedling, which method comprises the following steps:

providing a nutrient medium comprising particles of a solid component contained within a semi-solid component containing water and a carbohydrate nutrient for the embryo or germinant, said solid component being present at a concentration up to 10% (w/v);

dispensing a quantity of the nutrient medium onto a surface of a porous solid growth substrate for the somatic plant embryo or germinant to form a pool of said nutrient medium and contacting said plant embryo or germinant with said nutrient medium in such a manner

that said pool of nutrient medium provides said embryo or germinant with physical support to maintain a generally upright growth orientation; and

exposing said embryo or germinant to environmental conditions effective for growth into an autotrophic seedling;

wherein at least said dispensing, contacting and exposing steps are carried out *ex vitro* in non-sterile conditions; and

wherein said particles forming said solid component are adapted to remain in contact with said embryo or germinant after said semi-solid component undergoes dissipation in said environmental conditions, thereby providing continuing physical support for said embryo or germinant after said dissipation.